Genesis of life-threatening ventricular arrhythmias during the delayed phase of acute myocardial ischemia. Role of cellular electrical coupling and myocardial heterogeneities
de Groot, J.R.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
SYNTHESIS

Joris R. de Groot
Introduction.

Despite the improved treatments for myocardial infarction, sudden death resulting from acute myocardial ischemia still accounts for a considerable percentage of deaths in the industrialized world\(^1\). The mechanism of sudden death in this setting is ventricular fibrillation in the majority of cases\(^1,4\). In experimental animals, life-threatening arrhythmias occur in two distinct phases that are separated by a relatively arrhythmia free interval\(^2,7\).

Sofar, many studies were directed to the mechanism of lethal arrhythmias during the first 10 minutes of ischemia\(^8\), but the delayed phase of acute ischemia is relative terra incognita. From animal studies it is assumed that the delayed or 1B phase comes with an equal number or more lethal events than the early or 1A phase\(^5,10\), but mechanistic explanations for this phase of arrhythmias are restricted to the absence of diastolic bridging\(^5\) and the temporal association with cellular uncoupling\(^10,12\). Therefore, the aim of the studies presented in this thesis was to investigate the electrophysiological mechanism of delayed or 1B ventricular arrhythmias during acute ischemia\(^5,6\).

Cellular electrical uncoupling.

Acute ischemia induces metabolic changes, among which intracellular acidosis\(^13\), increase in cytosolic calcium concentration\(^1,14\) and the release of fatty acid metabolites\(^15,16\) that lead to changes in gap junctional conductance. Passive electrical properties of myocardium govern the spread of current through the myocardium and directly influence conduction velocity\(^11\). Therefore, it was assumed that cellular electrical uncoupling during ischemia is arrhythmogenic\(^17,18\) both because conduction slowing can occur\(^19,20\) and because very local heterogeneities can arise that give rise to microreentry\(^21\). Indeed, Smith et al. demonstrated that spontaneous VF occurred when tissue impedance started rising during ischemia\(^10\), and both processes are postponed after ischemic preconditioning\(^12\). We investigated the time window during which ventricular fibrillation can be induced by programmed stimulation\(^22\), and described in chapter 3 that ventricular fibrillation can occur in a time window that encompasses an increase in tissue impedance from zero to no more than 40% of its final value. Hence, only at moderate increase in tissue impedance, the arrhythmogenic substrate is present, and the arrhythmogenic substrate does thus not relate to complete uncoupling, but to a modest decrease in electrical coupling.

Very slow conduction velocity, as would be expected if heterogeneous cellular uncoupling takes place\(^19,20,23\), was however not observed, nor did we find evidence for microreentry. In addition, the time course of tissue impedance rise\(^24,27\), a measure for cellular uncoupling, is homogeneous within the ischemic zone, further corroborating the hypothesis that cellular electrical uncoupling does not cause heterogeneity at a cellular level.

To investigate further the mechanism by which cellular uncoupling causes intercellular heterogeneities, we performed the experiments that are described in chapter 6. Here we demonstrate that in isolated cardiac myocytes the intrinsic differences between cells can be held responsible for the duration of the uncoupling process as observed in multicellular preparations. Moreover, we demonstrate in electrically coupled cell pairs that no differences in action potential duration and time to ischemia induced rigor are present among the two paired cells, and that action potential duration in both cells remains the same up to the moment that cells are no longer excitable. Therefore, it can be speculated that quantitatively gap junctional conductance does not fall below 1.3 nS during ischemia\(^28\). Hence, cellular communication remains possible during the progress of ischemia, and therefore, the occurrence of local heterogeneities is very unlikely. Consequently, differences in functional properties should arise over larger distances and at a larger scale, and are not caused by
ischemia-induced cellular uncoupling at a cellular level. Instead, differences at a cellular scale are prevented by intact cellular coupling that persists longer than excitability.

No Microreentry.

Based on the prediction formulated above that microscopic differences at a cellular level are absent, and that arrhythmogenic heterogeneities are expected to arise over larger distances within the ischemic zone, we measured conduction velocity and wavelength in intact hearts to describe the minimal size of reentrant circuits during the delayed phase of ischemia.

The results in chapter 5 demonstrate that, albeit that conduction velocity in longitudinal and in transversal direction decreased during 60 minutes of ischemia, no examples of very slow conduction were found, and no microreentry occurred. In addition, the refractory period did not decrease to values less than 170 ms, which in combination with the measured conduction velocities lead to a minimal possible wavelength of 2.8 cm. Given the fact that for sustained fibrillation either multiple wavelets should be present or one single, rapidly moving rotor, either an extremely large ischemic zone or, more likely, the presence of the non ischemic tissue is required for the maintenance of the arrhythmia. Therefore, it is unlikely that microreentry occurs during ventricular fibrillation in the delayed phase of acute ischemia.

Next, we directly falsified the hypothesis that microreentry underlies ventricular fibrillation during the delayed phase of ischemia by attempting to induce VF in isolated parts of left ventricle. We used the same programmed stimulation protocol as described in chapter 3, that assured VF in 90% of the cases in that chapter. Our failure to induce VF in these experiments proves that microreentry cannot be the underlying mechanism of VF during the delayed phase of acute ischemia.

Macoreentry.

In the experiments in isolated left ventricular preparations that are described in chapter 5 we found that activation frequently ran around a line of functional activation block. In many cases, incomplete revolution of the activation front around a line or a region of block was observed, and occasionally complete reentrant revolutions were encountered. However, this arrhythmogenic substrate did not allow sustained arrhythmias. Contrary to this observation we demonstrated the induction of VF in the intact heart with three premature stimuli in 90% of cases and in 60% with only one single premature stimulus applied from within the central ischemic zone. Thus, we conclude that: 1) conduction velocity and refractory period do not decrease enough to be compatible with microreentry, 2) we were not able to induce ventricular fibrillation in isolated left ventricular preparations, 3) cellular electrical coupling remains intact up to the moment that cells are no longer electrically excitable during metabolic inhibition, thus no microscopic heterogeneities can arise (chapter 6). We must therefore conclude that macroreentry is the most likely electrophysiological mechanism underlying ventricular fibrillation in the delayed phase of acute ischemia. This hypothesis is strengthened by the observation that mostly planar activation fronts were observed during 1B-VF (chapter 3 and 4). Moreover, within the ischemic zone, distinct domains exist with the same dominant frequency of ventricular fibrillation, as has been described earlier in the normoxic heart, which suggests that within those domains electrophysiological properties are equilibrated through sufficient cellular electrical coupling.
Alternatively, non reentrant mechanisms underlie delayed ventricular arrhythmias\textsuperscript{34-37}, but our observation of activation front revolutions around lines of block does not support this option, and seems to give a sufficient explanation for the arrhythmogenic substrate.

**Ischemic subepicardium is the arrhythmogenic substrate.**

We suggested that the subepicardium overlying the ischemic zone is the region where the electrophysiological deterioration leading to malignant arrhythmias occurs. Chapter 3 describes experiments demonstrating that recovery of maximal negative slope of the extracellular electrogram takes place in the subepicardium, whereas in the midmyocardium sites become inexcitable, and that this recovery hallmarks the end of the delayed phase of arrhythmias. In chapter 4 we demonstrate that during this phase activation patterns of ventricular fibrillation become confined to the two dimensional layer of the ischemic subepicardium. We demonstrate that the arrhythmia becomes more stable, which is consistent with a decreased propensity to spontaneous occurrence. Meanwhile, in this study we did not observe recovery in action potential duration or in conduction velocity, which might have related to the absence of mechanical activity in the mechano-electrically uncoupled preparation\textsuperscript{38}. The observation that dispersion in action potential duration takes place without a change in mean action potential duration does indicate that locally both recovery and further deterioration takes place. After a dip after 15 and 30 minutes of ischemia, dominant frequency of ventricular fibrillation recovers to preocclusion values. These changes temporally coincided with the transition of activation patterns of ventricular fibrillation to the two dimensional structure of the ischemic subepicardium.

Hence, we suggest that the arrhythmogenic substrate is present while coupling of the viable subepicardium to the severely depressed midmyocardium causes electrotonic depression of the former\textsuperscript{39-42}. Progression of cellular uncoupling diminishes this electrotonic load and allows the subepicardium to partially restore its electrophysiological properties, despite that intramurally extracellular potassium concentration does not decrease\textsuperscript{6}. The fading interaction between midmyocardium and subepicardium is evident from the confinement of activation patterns during ventricular fibrillation to the two-dimensional domain. The figure graphically displays this novel mechanism for ischemic arrhythmogenesis. Panel A shows the interaction between the severely depressed midmyocardium and the viable subepicardium, as in figure 1.2 from the Introduction section. Because cellular coupling is partially intact (some gap junctions are closed (grey), and some are still open (white)), conduction velocity is decreased (meandering arrow) and action potentials are depressed within the subepicardium. Panel B shows the time course of the major determinants of electrophysiological depression in the subepicardium during 60 minutes of ischemia. Change in tissue impedance (dashed line), change in midmyocardial extracellular potassium concentration (adapted from\textsuperscript{38}), and the expected decrease within the subepicardium\textsuperscript{38} (stippled line), decrease in number of excitable sites (dV/dt\textsubscript{min} less negative than -2.5 V/s) are shown. The time window during which VF could be induced is shaded grey.

The failure to demonstrate diastolic bridging was earlier interpreted as the absence of reentrant activation\textsuperscript{9}, but can also be explained as a consequence of a too small number of electrically active subepicardial cells to cause visual changes in the body surface ECG. In addition, direct measurement of midmyocardial electrograms demonstrates that this tissue becomes fully inexcitable (chapter 3), which makes intramural reentry\textsuperscript{6,44} an unlikely causal mechanism for 1B arrhythmias.
Figure 8.1A Schematic of a piece of left ventricular myocardium excised from the ischemic zone, as in figure 2 from the Introduction. Subepicardium is electrotonically depressed through partially intact cellular coupling (some gap junctions are closed (grey), others are still open (white)) to the severely depressed ischemic midmyocardium.

B. Schematic of the time course of proposed interactions affecting subepicardial electrophysiology during 60 minutes of ischemia. Course of extracellular potassium concentration $K^+$ in midmyocardium (black line) and the expected decrease in subepicardium (stippled line), tissue impedance ($R_t$, dashed line), transversal wavelength ($\lambda$, circles) and the number of excitable subepicardial sites (ESS, diamonds) are drawn. The shaded area represents the time window during which VF can be induced with programmed stimulation.

Mechanical factors induce delayed ventricular arrhythmias.

For an arrhythmia to occur, both an arrhythmogenic substrate and a trigger should concur. Pogwidz and Corr have demonstrated that both reentrant and non-reentrant mechanisms underlie premature depolarizations that induce ventricular tachycardia and fibrillation. During the early or 1A phase, systolic potential difference between ischemic and non-ischemic cells cause a so-called current of injury flowing from the ischemic towards the normal myocardium, that can be large enough to induce premature depolarizations. Mechanical factors have also been described to induce premature depolarizations and to lower the threshold for arrhythmias. Indeed, mechanical interactions between the contracting non-ischemic tissue and the quiescent or contractured ischemic zone exerts a force on the border between these two compartments. Notably, this was the location where we found the focal occurrence of premature beats. The association between mechanical interactions at the ischemic border zone and the occurrence of arrhythmias is further strengthened by the observation that in working preparations (both the in situ beating and loaded Langendorff perfused hearts) significantly more premature beats occur compared to isolated non loaded hearts. Consequently, the arrhythmogenic triggers should not arise in hearts that are mechanically uncoupled. Indeed, in preliminary experiments we found that significantly less triggers arose in hearts that were loaded with DAM. The fact that the use of DAM postpones the onset of cellular uncoupling (the substrate) is principally not related to the mechanism of the trigger, and has therefore no particular meaning for the proposed mechanism.

Clinical implications.

Sudden cardiac death presents a particular logistic problem for preventive strategies. Once it occurs, immediate defibrillation can restore sinus rhythm. Consequently, the incidence of death from ventricular fibrillation in the setting of acute ischemia is extremely low in the setting of the coronary care unit. However, if it occurs outside the hospital, the chance to leave the hospital alive after cardiac arrest, decreases exponentially with the duration of absence of circulation. Also the duration of cardiopulmonary resuscitation is associated with detrimental outcome. Thus, once professional help is available and specific antiarrhythmic drug treatment can be administrated, also defibrillation can be performed which would make other treatments superfluous.

Preventive strategies for sudden death should be directed to simple interventions that the patient or his direct bystanders can perform, comparable with the use of nitroglycerin in anginal symptoms. Chronical medical therapy is undesirable: the effects of lowering the number of arrhythmogenic triggers with antiarrhythmic drugs have been disappointing: many studies report an
excess in mortality in the treated groups, because the antiarrhythmic agents used all have a proarrhythmic component. The beneficial effect of beta blockade during the acute phase of myocardial infarction seems to be caused by reduction of cardiac rupture, rather than by an antiarrhythmic mechanism. Inhibiting mechanical activity of the heart, which would be the most rational choice to reduce the number of arrhythmogenic triggers based on our results described in chapter 7, is undesirable in patients. An implantable defibrillator, although useful in high risk populations, is not an option for the general population, let alone the fact that the individual risk for sudden death is often not known. The increasing availability of cardiac defibrillators in the public domain, in airports and train stations and in the armature of police cars can possibly add to more beneficial outcomes of resuscitation.

We can speculate that specific interventions that decrease gap junctional conductance in the setting of acute ischemia, might shorten the temporal window during which delayed ventricular arrhythmias can occur, because their arrhythmogenic substrate depends on electrotonic interaction between viable subepicardium and depressed midmyocardium. The rationale for such an intervention is in the first place to prevent ventricular fibrillation and sudden death, because although direct defibrillation is successful in more than 95%, it still fails in few percent of the cases (R.W. Koster, personal communication). In the second place, chest compressions during resuscitation and electrical defibrillation have been associated with increased levels of creatine kinase MB and troponin T. The underlying mechanism is probably related to contusion of the heart and chest muscles, but also electroporesis, leading to cell damage through calcium overload, has been described. Thus, even if defibrillators are around, it is worthwhile to prevent their use.

The criteria to which such a couplolytic drug should adhere relate to the fact that they 1) should be easy to use as are the medications for obstructive pulmonary disease and that 2) their action is reversible in myocardium that can be salvaged upon reperfusion of the ischemic area. Potentially, inhalation of a modest amount of halothane, a gas used for anaesthesia which decreases gap junctional conductance, could be used for such purposes. However, the clinical applicability of such therapies needs to be investigated.

Conclusions.

The principal conclusions of the studies presented in this thesis are:

1) Delayed, ischemia-induced ventricular fibrillation can be induced within a temporal window that encompasses the rise in tissue impedance from zero to 40% of its final value.
2) Tissue impedance rises homogeneously within the ischemic zone.
3) Uncoupling of the ischemic subepicardium from the severely depressed ischemic midmyocardium hallmarks the end of the period that delayed ventricular arrhythmias can be induced and
4) Transition of the activation patterns during ventricular fibrillation from the three- toward the two-dimensional domain makes the arrhythmia more organized and stable.
5) After an initial decrease, dominant frequency of ventricular fibrillation recovers to pre ischemic values, but the number of domains with a distinct dominant frequency increases.
6) Mean action potential duration within the ischemic subepicardium decreases and does not recover, although increased dispersion of action potential duration indicates local recovery as well as local deterioration.
7) Duration of cellular coupling can be explained from intrinsic metabolic differences among cells, resulting from differences in anaerobic reserve.
8) Cellular electrical coupling remains intact up to electrical inexcitability.
9) Microreentry does not underlie ventricular fibrillation during the delayed phase of acute ischemia.

10) The trigger for delayed ventricular arrhythmias relates to mechanical interactions between the non-ischemic and the ischemic tissue.

References.


17. Saffitz JE, Yamada KA: Do alterations in intercellular coupling play a role in cardiac contractile dysfunction? *Circulation* 1998;97:630-632


25. Plonsey R, Barr R: The four-electrode resistivity technique as applied to cardiac muscle. *IEEE Transactions on Biomedical Engineering* 1982;29:541-546


49. Lab MJ: Transient depolarization and action potential alterations following mechanical changes in isolated myocardium. *Cardiovascular Research* 1980;14:624-637


51. Carmeliet E: Cardiac ionic currents and acute ischemia: from channels to arrhythmias. *Physiological Reviews* 1999;79:917-1017


